

## Characterization of a New Enterococcal Gene, *satG*, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the *ermB* gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase SatA (9). *Enterococcus faecium* isolates with *satA*-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant *E. faecium* UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin (MIC ≥ 16 µg/ml) and virginiamycin M (A compound; MIC, 16 µg/ml), whereas the

MIC of each antibiotic for the recipient was 1 µg/ml. PCR amplification for the *satA* gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (Vat, VatB, and VatC [1-3]) and (ii) efflux due to an ABC transporter (Vga and VgaB [4, 5]). PCR amplification for the *vat*, *vatB*, *vatC*, and *vga* genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, satI and satJ, have been made, producing a 144- to 147-bp fragment for *vat*, *satA*, and *vatB* (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of EcoRI-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

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RBS
1 cggtaaccgg ggatcctcta gactataatt aaaattaaat aactcaattc ggagggtacta
      start   primer satI
61 adcgtactat acgtgacgca aatgcaatct atccataactc agccatcaagagggtgtt
      M T I P D A N A I Y P N S A I K E V V F
      aa-motif I

121 ttatcaagaa cgtgatcaaa agtccaaata ttgaatttgttgg ggactacacc tattatgtat
      I K N V I K S P N I E Y G D Y T Y Y D D
181 acccgataaa tcccacccat tttgagaaac acgttaccaa tcactatgaa ttcttaggcg
      P V N P T D F E K H V T H H Y E F L G D
241 acaaattaat catcggtaaa ttttgttcta tcgccagtgg cattgaatttatcatgaacg
      K L I I G K F C S I A S G I E F I H H G
      aa-motif II

301 gtgccaaacca cgtaatggaaa ggttatttcga ctatccatt taatattttt ggtggcgatt
      A M H V M K G I S T Y P F N I L G G D W
361 ggcacacata cactctgaa ctgactgatt tggcgttcaa aggtgataact gtatgcggaa
      Q Q Y T P E L T D L P L K G D T V V G H
      aa-motif III

421 atgacgtgt gtttggggca aatgtgaccg tcctaccagg cgtaaaaata ggtgacgggt
      D V W V G Q N V T V L P G V K I G D G A

481 ccattatcgg agcaaatagt gttgtaacaa aagacgtcgc tccatataca attgtcggtg
      I I G A N S V V T K D V A P Y T I V G G
      primer satG2
541 gcaatccaaat tcaactcatc ggaccaagat ttgacccgga agttattcaa gcattagaaa
      N P I Q L I G P R F E P E V I Q A L E N
601 atctggcatg gtggataaaa gatattgaat ggtaaactgc taatgttctt aaactaatgc
      L A W W N K D I E W I T A N V P K L M Q
      stop

661 aaacaacacc cacacttcaa ttgataaaca gttaatgga aaaatgttaaa caaaaaagcc
      T T P T L E L I N S L M E K
721 gtcaagcaa tccaaaaatg attgtttaca cggcctttac tatttagtga atccaattta
781 ttaataatag atatgatata ccagaaaaaa atacactagc cacctctggc ggtactctac
841 tcgtatattt tatttacgac cttctgtatc taaaggtcac tccccgtcc ccagaaaata
901 aagc

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FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 aa which shows significant homology with other streptogramin acetyltransferases (aa motifs I, II, III; see also Fig. 2). The locations of the primers satG1 and satG2, specific only for the *satG* sequence, are underlined (plus strand).

		Motif I	
SatG:	1	M-----TIPDANAIYPNSAIKEVVFIRKNI-KSPNIEIGDYYDDPVNPTEFKVTHHYEFLGDKLI	63
VatB:	1	MK----YGPDPNSIYPMRERIKSVCPIRKNTI-TNPNIILVGDITTYCDVNGAERKPEEVTHHYEPRGDKLV	64
VatC:	1	MKWNQNQGPNPPEIYPIECNKGKVQPIKPSI-TKPNILWGVITSTYTDISK-DGESPESQVLYHYELGDKLI	67
SatA:	1	M-----GPNNPDDONPIECNKSVPQPIKPILEKLNVVEPGLITSTYTDISK-NOETPDQKLYHYPILNDKLK	62
Vat:	1	MNLNNNDHGPDPENILPIKGNRNLQPIKPTI-TNENILWGVITSTYDSKRGES-PEDQVLYHYEVIGDKLI	67
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		Motif II	
SatG:	64	IGKPCSIASGIBPFIDLWVINGISTYPNIIQGDWQYTPEL-TDLPLKGDTVGCIDWVIGDNV	128
VatB:	65	IGKPCAIAGIPIFIDLWVINGISTYPNIIQGDWQYTPEL-EDLPPFKGDTVGCIDWVIGDNV	129
VatC:	68	LGKPCSIGPGTTIICLWVLDLWDG-STPPPNLPGNGWEKHTPTL-BDLPYKGNTIEIQDWDVIGRDV	131
SatA:	63	IGKPCSIGPGVTIICLWVLDLWDG-STYPPNLPGNGWEKHMPLK-DQLPIKGDTIIGDWDVIGRDV	126
Vat:	68	IGKPCSIGPGOTTIICLWVLDLWDG-STYPPNLFRNGWEKYMPSL-KDLPLKGDTIIGDWDVIGRDV	131
***			
		Motif III	
SatG:	129	TVLPGVKIGDGAIIGANSVUTKDVAPTYIVGGNPIOLIGPRLPEPEVIALENLAW	183
VatB:	130	TVMPGIQIGDGAIVAANSVUTKDVPPTYTRIICGCPSPRIKKRPEDELIDYLLQIKW	184
VatC:	132	TIMPVGKIGDGAIIAAKSVVTKNVDPYSVGGNPSRLIKIRPSREKIAALLKVRW	186
SatA:	127	VIMPGVKIGDGAIVAANSVUVDKIAPYMLAGGNPANEIKQRFDQDTINQLLDIKW	181
Vat:	132	TIMPVGKIGDGAIIAAKSVVTKNVDPYSVGGNPLKPIRKPSQDVIEEWLALQW	186
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SatG:	184	WNKDIEMITANVPKLMQTPTLELINSLMEK	214
VatB:	185	WDMSAQKIFSNLTLCS--DLEKIKSIRD	212
VatC:	187	WDLDEISTINENI	198
SatA:	182	WNNPIDINENIDKILDNSIIIREVI	206
Vat:	187	WNLDMMKIINENLMP	199
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FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1–3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs I, II, and III—are boldfaced. Primers satI and satJ have been designed on the basis of the corresponding nucleotide sequences in motifs II and III (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated SatG (Fig. 2). Based on the sequence for *satG*, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (*E. faecium*, *E. hirae*, and *E. durans*) revealed the existence of the *satG* gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfopristin-resistant *E. faecium* (QDREF) isolates from hospitals in Germany, 9 were positive for *satG*. The high number of *satG* QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

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